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20, 1910, is hereby promulgated and shall remain in force until otherwise ordered:

> Vessels from ports in South America and the West Indies, Africa, Russia, China, India, and the Pacific Islands, while lying in United States ports, shall have all lines or hawsers leading to wharves or shore, protected by rat guards (for description see p. 907, Public Health Reports, June 7, 1912), and all gangplanks shall be raised at night unless men be placed nearby to destroy escaping rats.

FRANKLIN MACVEAGH,
Secretary.

THE TECHNIQUE OF THE LABORATORY EXAMINATION OF RATS FOR PLAGUE.

Being a condensation of an article on "Plague Infection in Rats," by GEORGE W. MCCOY, Passed Assistant Surgeon, Public Health Bulletin No. 30, "The Rat and its Relation to the Public Health."

The clinical manifestations of plague in rats are of little importance. It is generally said that the plague-infected rat staggers about with a drunken gait, loses fear of its natural enemies, and is readily captured. The experience with artificially infected rats indicates that the animals show no marked manifestations of illness until shortly before death when they become quiet, crouch in the corner of the cage, and try to hide.

As plague is a disease that gives rise to such characteristic gross pathological lesions in man and in laboratory animals, it is but reasonable to expect that equally distinctive lesions would be found in the rat, and this we find to be the case.

As to the comparative value of microscopical and macroscopical methods of diagnosis, the Indian Plague Commission (3) states that:

> The results of tests carried out for the purpose of comparison make it manifest that the naked eye is markedly superior to the microscopical method as an aid in diagnosis, and as the result of our experience we are prepared to make a diagnosis of plague on the strength of the macroscopical appearances alone, even though the other results of cutaneous inoculation and culture are negative and the animal shows signs of putrefaction.

To one who is acquainted with them, these lesions are as characteristic as those of any infectious disease in man. It is quite true that occasionally atypical cases are encountered where the majority of the gross lesions are wanting, and in such cases it becomes necessary to resort to the inoculation of animals or to cultural investigations in order to make a diagnosis. Such cases are, however, if anything, rarer than are atypical post-mortem findings in pneumonia or in typhoid fever in man.

MODE OF EXAMINATION.

The mode of examining rats for plague infection is as follows:

Rats are immersed in any convenient antiseptic to kill fleas and other ectoparasites that might carry infection from the plague-infected rat. They are then nailed to a shingle by an attendant. Another attendant reads off the address on the tag attached to the rat, puts a check number on the shingle, and records the address and check number on the card shown on page 1187. After being checked, the rats are dissected by reflecting the skin from the whole front of the body and neck so as to expose the cervical, axillary, and inguinal cavities, and by opening the thoracic cavities with scissors. After examination by the medical officer, they are removed from the shingle and any plague-infected rats burned as soon as the necessary investigation has been made. Careful search for buboes should be made in the regions of the various peripheral lymph glands, and the abdominal and thoracic organs subjected to careful scrutiny. This work should be done in a rat-proof, well-lighted building provided with water, gas, and sewer connections. The utmost care should be taken to avoid any undue risk of infection. The wearing of rubber gloves is not necessary. Everyone who has to handle infected animals must be sufficiently alive to the danger of infection.

In the extensive work conducted by the Indian Plague Commission (3) attendants were protected with Haffkine's prophylactic. This is undoubtedly a wise precaution and should be taken if possible.

A lead-topped worktable which slopes gently from the sides and ends toward the center, where a drainpipe leading to a vessel containing a disinfectant is attached, is very useful.

The layman of average intelligence readily learns to recognize the gross lesions of rat plague and it is wise to train laboratory attendants to do this. Every rat should be subjected to careful scrutiny by the physician responsible for the work. The majority of rats may be put aside after a cursory examination as entirely beyond suspicion of infection. Probably 8 or 10 per cent of them will require a very careful examination for the gross lesions of plague. A card which has been found useful for keeping records of suspected and infected animals is shown on page 1187.

THE GROSS LESIONS OF NATURAL RAT PLAGUE—ACUTE PLAGUE.

SUBCUTANEOUS INJECTION.

This is the sign which usually first attracts attention. White (4), in discussing plague in rats, states that "the most noticeable post-mortem appearance of the plague rat is the engorgement of the subcutaneous blood vessels, together with a diffuse pink color of the subcutaneous muscles, which have a peculiar dry, waxy translucency." It is frequently the case to have an attendant who is dissecting rats remark that he had found an infected rat after the first incision was made in reflecting the skin. The injection is dark red, and upon close inspection the small vessels are seen uniformly distended with blood. It is usually distributed over the whole surface of the body, but occasionally it is confined to the side of the body on which the primary bubo was found. A bright pink injection is a rather common finding, but it is not likely to be mistaken for the injection of plague infection. Subcutaneous edema, confined to the vicinity of the bubo, is occasionally encountered.

In San Francisco an injection identical in appearance with that found in plague infection was found only twice, and in each case there was associated with it a small discharging subcutaneous abscess. There were no other lesions in either case, and the pus from these abscesses failed to produce plague in guinea pigs.

In a series of 61 consecutive plague rats in San Francisco, injection was present fifty-two times; it was confined to the region of the bubo twice; it was unilateral twice; and was general in distribution forty-eight times. It was slight thirteen times, moderate fifteen times, marked sixteen times, intense eight times.

THE BUBO.

This is the most reliable single sign of plague infection, and when present in typical form is sufficient evidence on which to base a diagnosis which rarely proves erroneous.

The gland involved is usally surrounded by a more marked injection than is present elsewhere, and an infiltration which at times is hemorrhagic. This surrounding hemorrhage which was common in the plague rats described by the Indian Plague Commission was met with very rarely in San Francisco. The gland proper is usually caseous. The contents may be shelled out very readily, though prior to section the gland feels very firm. In the cases seen in San Francisco, the contents of the buboes were recorded as being hemorrhagic four times and as caseous twenty-nine times. Pestlike bacilli were noted as present in 18 cases, in 6 of which the "coccoid" form predominated. They were recorded as absent five times.

Indolent enlargement of the lymph glands is very commonly encountered in rats that are not infected with plague. Among old rats probably 15 per cent will show this. Such glands, however, are tough, elastic, and not surrounded by infiltration. They are not likely to be mistaken for the plague buboes. In the leprosy-like disease of rats, the glands may reach an enormous size.

Observers differ as to the location of the primary bubo. Skschivan (1) states definitely the location of five primary buboes in plague rats seen in Odessa in 1901. Two were in the axilla, two in the inguinal region, and one in the neck. Kitasato (5) says:

> To judge from the experience of the past, it can be suggested that in examining rats particular attention should be paid to their submaxillary and cervical glands and to the spleen. These organs, in most cases, show the evidence of infection, if there be any.

From this it would appear that he regarded the neck glands as the most frequent seat of the bubo. It may be remarked here that his experience was derived from plague rats seen in Asia.

There was a marked difference between the experience in San Francisco and that in Bombay. This is demonstrated in the following table, which shows the location in percentage of single buboes in each situation:

	Neck.	Axilla.	Groin.	Pelvis.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Indian Plague Commission, Bombay—2,923 rats (3)	75	15	6	4
Wherry, Walker, and Howell, San Francisco (6)—8 rats	12	12	75	....
Federal laboratory, San Francisco—32 rats	....	22	72	6

The American figures are too small to be of much significance, but one is struck with the fact that in Bombay three-fourths of the buboes are in the neck, while in San Francisco three-fourths of all found are in the inguinal region. We have records of only three multiple buboes found in rats in San Francisco, and in no case was either of the buboes in the neck; while in Bombay, to quote from the report (3), "Of the rats with multiple buboes, 54.5 per cent had a bubo in the neck." Striking as these figures are, there is further evidence that the inguinal region is the commonest location of the bubo in plague rats in this vicinity.

Particular care was taken to look for cervical buboes, as it seemed rather inconsistent to find the other lesions so fully in accord with those found in India, yet to have the location of the bubo to differ so radically. We have not encountered a mesenteric bubo in our work in San Francisco. The Indian Plague Commission found none in over 5,000 naturally infected plague rats. As mesenteric buboes are very commonly encountered in plague infection brought about by feeding, they conclude that the absence of these buboes in naturally infected rats is strong evidence that the infection does not enter by the alimentary canal.

THE GRANULAR LIVER.

Two lesions of the liver are encountered in plague rats. The one most frequently observed is spoken of by the Indian Plague Commission as "fatty" change, though it is explained that this term refers to the naked eye appearance, as microscopically the lesion is found to be due to a necrosis of the liver tissue. When this change is present the organ is found to be rather yellowish in color and is studded with an enormous number of yellowish white granules which are about the size of a pin head. This lesion, which was very common in the San Francisco cases, is very readily recognized.

The other lesion is a marking of the organ with grayish white spots; "they are typically of the size of a pin's point, and give the surface of the organ a stippled appearance as if dusted over with gray pepper" (3, p. 331). This appearance, which is less frequently encountered than is the preceding one, is more difficult to recognize; indeed the most careful scrutiny is necessary to avoid overlooking it.

Rats that have been fed with certain biological preparations used to destroy rodents (Danysz's virus and similar preparations) often present lesions in the liver resembling those due to plague infection. The granules are, however, larger and more distinct. In these cases the spleen is enlarged and generally granular, but rarely dark and friable, as in plague infection.

THE SPLEEN.

The size of the spleen of healthy rats of the same weight varies so greatly that often one can not be sure as to what constitutes an enlargement of this organ.

In plague rats this organ is markedly enlarged, firm, friable, rather dark in color, and occasionally presents small granules under the capsule. As Skschivan (1) pointed out, these granules are not encountered as often as are granules in the liver. At times the organ presents a very distinctly mottled appearance. This latter appearance is much more frequently seen in artificially inoculated rats than in those found infected in nature. The organ is occasionally distinctly slate colored.

PLEURAL EFFUSION.

This sign of rat plague is of great importance when associated with other suspicious lesions. The effusion is bilateral, and is serous in character, usually clear, though it is occasionally bloodstained. Pleural effusion is rarely found in rats other than those that are plague infected. The following table shows in percentage the frequency of the various macroscopical lesions of acute natural rat plague, as observed in Bombay and in San Francisco:

	Subcutaneous injection.	Bubo.	Granular liver.	Large dark spleen.	Pleural effusion.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Indian Plague Commission, Bombay—4,000 rats	69	85	58	....	72
Wherry, Walker, and Howell, San Francisco—88 rats.	59	14	14	68	71
Federal laboratory, San Francisco—62 rats	85	57	87	74	59

It is recognized that the data from the San Francisco records is so much smaller than that from the Indian report that perhaps no just comparison is to be made. However, the figures are quite similar, except for the small percentage of buboes and of liver lesions in the work of Wherry, Walker, and Howell. The work of these observers was done in the early part of the epizootic in San Francisco while the other figures from that city are drawn from records later in the campaign. No single sign is pathognomonic.

It is a combination of two or more of the signs that is of moment. The subcutaneous injection with a typical liver or these signs associated with a typical spleen afford good grounds for a diagnosis. A rat showing a typical liver associated with a pleural effusion will usually prove to be plague infected, and if a large, dark, firm spleen is also found a diagnosis may be considered as practically established.

As has been pointed out by several writers gross lesions of plague may be distinguished even in rats that are badly decomposed.

CHRONIC PLAGUE.

No case of natural chronic plague has been encountered in San Francisco. Only one case was found among the many hundreds of plague rats examined by the Indian Plague Commission (3, p. 457) in Bombay. However, this commission encountered a considerable number of cases among *Mus rattus* in the Punjab villages of Kasel and Dhand. The lesions were purulent, or caseous foci. They classify these cases as follows: Chronic plague of the visceral type, which is further subdivided into splenic nodules and abscesses, and mesenteric abscesses; chronic plague of the peripheral type in which abscesses are situated in the regions of the peripheral lymph glands.

Plague bacilli were either absent or very scanty upon microscopical examination. They were, however, quite frequently recovered by cultural methods, and in the great majority of the cases the organisms were fully virulent. No evidence was forthcoming to show that this chronic rat plague had anything to do with the recurrence of acute plague among the rats.

We have diligently sought for chronic plague among the rats in San Francisco, but, as we said above, without success, although a considerable number of lesions that correspond perfectly to the description of chronic plague have been submitted to the guinea-pig inoculation test, but invariably with a negative result. An account of the lesions of chronic plague as observed among inoculated rats is given in another part of this paper.

Pound (7) believes that recovery from plague in rats is shown by the presence of pigmented lymphatic glands. Kister and Schumacher (2) mention pigment deposits in the inguinal region, but remarked that they are not characteristic of plague, a view which McCoy believed was correct, as he frequently saw them in San Francisco among the older rats, in which there was no reason to suspect previous plague infection, and they were almost uniformly absent in rats that were experimentally infected with plague.

RAT PLAGUE WITHOUT GROSS LESIONS.

Plague infection may be present in a rat without bringing about any recognizable gross lesions. For example: Dunbar and Kister (8) mention a rat, which came from a ship on which plague rats had been found, that had no lesions, and cultures were negative; but a guinea pig cutaneously inoculated died of plague.

Among a considerable number of inoculated rats perhaps once or twice in a hundred cases there was found nothing at the post-mortem examination that would suggest plague infection, yet cultures or inoculation of guinea pigs would demonstrate the presence of *B. pestis*. Such cases are very infrequent, but it should be kept in mind that they do occur. When a large number of rats are to be examined it would be impracticable to inoculate a guinea pig from each rat; and even if one did this the occasionally resistent guinea pig would introduce a larger error than exists by placing dependence upon the gross lesions for a diagnosis.

MICROSCOPICAL EXAMINATION.

The exact weight to be given to the morphology of the organisms found in smears from the organs of a rat suspected of being plague infected is a matter of individual judgment. Smears from a bubo and from the spleen may show no organisms at all, or none even remotely resembling *B. pestis*, and yet by culture and inoculation methods we may be able to demonstate that the animal is plague infected. Attention has been called to this point by several observers and every worker in this field has the experience sooner or later.

In other cases the smears will show such numbers of perfectly typical bipolar bacilli and "involution" (coccoid) forms as to leave scarcely any doubt as to the nature of the organism. But even here cases that are not plague are encountered that will deceive even the most experienced. We have been accustomed to put great dependence on the "coccoid" forms of the organism, but late in the San Francisco experience smears from a splenic nodule that was not regarded as due to plague showed perfectly typical "involution" ("coccoid") forms. Animal inoculations and cultures showed that the tissues contained no plague bacilli.

In addition to these two classes of cases we have a third, where smears show a few typically shaped bacilli, or where a considerable number of typical-looking bacilli are found along with many other bacterial forms. There is no safe rule for reaching a conclusion in these cases, and one must resort to culture or to inoculation methods, or both. In any such case it is always a good plan to let the macroscopical findings have more weight than the miscroscopical.

The bipolar appearance of *B. pestis* is so largely dependent upon the technique of staining, fixing, length of time the stain is allowed to act, and the length of the washing that it should never be given great weight. Here, as elsewhere in bacteriology, many errors are to be avoided by not depending too much upon the morphology of the organism under investigation.

BACTERIOLOGICAL DIAGNOSIS OF RAT PLAGUE.

While the gross lesions of rat plague are often sufficiently characteristic to justify a positive diagnosis, and the gross lesions in conjunction with the miscroscopical examination will in other cases enable us to say definitely that a rat is plague infected, still a certain number of cases occur in which it is necessary to resort to other methods, and there are circumstances, such as the first case in a community, that make a complete bacteriological confirmation of a diagnosis necessary.

B. pestis may often be isolated in culture from the tissues (bubo, liver, spleen, or heart's blood) of an infected rat. Unless the tissues are badly contaminated with other organisms, plate or stroke culture will yield a growth of *B. pestis* in pure culture, or isolated pestlike colonies may be transferred to other media.

It is unwise, however, to trust to cultural methods alone. In the majority of doubtful cases it is advisable to inoculate guinea pigs or white rats. The lesions of plague in these animals are quite characteristic, and *B. pestis* may readily be recovered from their tissues if cultures are made at once after death.

A pure culture of the organism under suspicion is obtained from the naturally infected animal or from a laboratory animal inoculated from the one under suspicion. This culture is studied in regard to its morphology; first, on agar, where it grows as a short rod, or often in the shape of a coccus; second, in broth, where it often grows in streptococcuslike chains; third, on agar containing 3 per cent sodium chloride, where most extraordinary alterations in morphology occur, giving large balloon-shaped bodies, objects resembling gigantic cocci and enormous trypanosome-shaped forms, the so-called "involution" forms. These involution forms must not be confused with the so-called "involution" (coccoid) forms of the organism found in smears from animal tissues.

The great diagnostic value of involution forms developed when *Bacillus pestis* is grown on salt agar should be noted. No other organism gives forms that are at all likely to be mistaken for those of *Bacillus pestis*, except *B. mallei*, and of course the other points of difference would at once serve to distinguish the latter organism.

B. pestis is Gram negative, though this point is of no great value except to distinguish the "coccoid" forms from pus cocci.

The appearance and character of the culture should be as follows:

Agar.—Smooth, glistening, round, whitish colonies, which are found to be sticky when touched with an inoculating needle.

Broth.—A scanty surface growth which falls, often in globular masses, when the tube is gently agitated; and a fine flocculent precipitate.

Litmus milk.—Generally rendered slightly acid.

Glucose broth.—Rendered slightly acid. Gas is not formed.

Lactose broth.—Unchanged in reaction. Gas is not formed.

The other cultural reactions are of no material assistance in the identification. In routine work the appearance of the growth on agar and in broth, together with the involution forms on salt agar, is sufficient for identifying the organism.

The plague bacillus is a nonmotile organism, a point worth bearing in mind.

A culture answering the above description when rubbed into the shaven skin of a guinea pig or a white rat should cause the death of either of these animals of plague within 10 days, and an organism must be isolated from their tissues after death corresponding to the one inoculated.

If one wishes to be doubly certain, one may inoculate a series of laboratory animals, giving to half of them a sufficient dose of antipest serum. The protected animals should recover, or markedly outlive the controls, which should die in the usual time.

As to the virulence of cultures of the bacillus from cases of rat plague Klein (17) states "that *B. pestis* bred in the rat is of decidedly less virulence than that bred in the human subject; moreover, the former is liable, outside the animal body, to a much greater extent to rapidly lose its virulence." It is evident that in any given epidemic it will be very difficult to say just which strain, rat or human, one is dealing with.

In the case of the strains of *B. pestis* recovered from rats in San Francisco we have seen nothing to justify such an opinion as Klein expresses. The cultures are all highly virulent and retain their virulence under artificial cultivation.

The value of inoculation by the cutaneous method to demonstrate the presence of plague infection in putrefying tissue is well known.

Kolle and Martini (9) compares the cutaneous method of inoculation to the use of an agar plate in separating plague bacilli from other organisms, and so regularly does *B. pestis* penetrate the skin and infect the animal, and so rarely do other organisms do this, that it offers a certain and accurate method of "filtering out" *B. pestis* from any badly decomposed tissue.

The technique of the cutaneous method of inoculation, or "vaccination," as it is sometimes called, is very simple. An area about an inch square is shaven on an animal's belly, taking care to abrade the epithelium slightly. The culture or suspected tissue is rubbed on this shaven area with a platinum loop or a dressing forceps. Guinea pigs when inoculated in this manner generally die before the seventh day; white rats die a day or two earlier.

Kister (10) uses a drop of juice from an organ rich in bacilli for agglutination experiments with antipest serum. This would appear in many cases to be of very material assistance, and the objection that it is difficult to form a uniform emulsion of the bacteria would be avoided. The well-known tendency of *B. pestis* to grow in clumps in culture is the main reason why agglutination reactions have not been more extensively used in plague work.

Skschivan (1) makes use of Pfeiffer's phenomenon in establishing the identity of a given organism as *B. pestis*.

To assist in the early diagnosis of plague, Dunbar and Kister (8) practiced intraperitoneal inoculation of laboratory animals and used a parallel series of immunized animals. As is well known, intraperitoneal inoculation with plague cultures or infected material leads to the early death of the inoculated animal, and it is evident that the survival of the immunized animal would afford considerable evidence that the material used for inoculation contains *B. pestis.*

PEST-LIKE BACTERIA FOUND IN RATS.

The somewhat general impression that there are a considerable number of organisms that are readily mistaken for *Bacillus pestis* is not justified, provided one gives attention to cultural and inoculation investigations. It is quite true that there are a considerable number of organisms which in smears from tissues are scarcely to be distinguished *morphologically* from *B. pestis.* The similarity, however, usually ends there. A few resemble plague somewhat closely in cultural reactions, and especially *B. pseudotuberculosis rodentium* (Pfeiffer) should be mentioned here; but these differ in pathogenicity. For example, the above-named organism is not pathogenic for rats.

Neumans (11) reviews the subject of pest-like organisms pathogenic for rats, and describes an organism belonging to this group which he isolated from the body of a rat. His work clearly shows that none of the organisms that have been described should cause any serious difficulty in the hands of a careful investigator.

Kister and Schmidt (12) describe an organism closely resembling *B. pestis* in many respects, and with which guinea pigs could be successfuly infected by the cutaneous method. This organism, which was also pathogenic for rats and mice, belongs to the hemorrhagic septicemic group. It differed from *B. pestis* in that it gave no involution forms when grown upon salt agar and was much more rapidly fatal to laboratory animals.

Augeszky (13) observed an epidemic among gray rats in his laboratory which was due to a pest-like organism belonging to the Friedlander group. The animals died after a couple of days of illness. At the post-mortem examination the spleen was found large, soft, and congested. There was a hyperemia of the intestines, lungs, and liver. In the spleen were found many, and in the heart's blood few, capsulated bacilli, some of which resembled *B. pestis.* The cultural reactions were in nowise similar to those of *B. pestis.* He found that inoculation of rats with a pure culture of this organism sometimes killed in as short a time as 24 hours, sometimes as late as two or three weeks, and in some cases the lesions were not very unlike those sometimes produced by *B. pestis.* However, this organism by its different cultural reactions, and the fact that the capsule is usually easily demonstrated, would probably never be a source of any confusion.

ARTIFICIAL INFECTION OF RATS WITH PLAGUE.

For laboratory purposes in general it is customary to use tame white rats, and in plague work they are especially satisfactory, as they are easily handled, rarely harbor fleas, are very susceptible to the infection, and finally and most important, they frequently die a day or two earlier than guinea pigs. At times it may be necessary to use wild rats on account of a failure in the supply of white rats or for the sake of economy. This may be done very satisfactorily, if one bears in mind the fact that a considerable number of wild rats are more or less immune to plague infection, especially when the infectious material is introduced by Kölle's (cutaneous) method. Therefore it is always advisable to use three or four wild rats where one white rat would be sufficient. They should be kept in a container of such design that there is no possibility of their escaping. The inoculation is best conducted with the animal under the influence of ether.

MODES OF INFECTION.

Rats may be infected experimentally by the ingestion of contaminated material and by the application of virulent plague bacilli to a mucous or a cutaneous surface or by subcutaneous injection of the organism.

Practically we may confine our study to inoculation by the cutaneous method and to subcutaneous inoculation when the material is injected in the ordinary manner. A useful modification of the latter method is to make a small pocket under the skin of the abdomen and thrust the suspected material into this pocket. This avoids the necessity of making an emulsion of infectious matter, such as the organs of an animal. The time that elapses between the inoculation of a rat with virulent culture of plague bacilli and its death varies somewhat with the size of the dose and with the mode of inoculation.

The *lesions* found, when an artificially inoculated rat is examined after death, are in a general way similar to those found in naturally infected rats with certain differences to be mentioned later.

LOCAL REACTION.

The most striking difference between natural and artificial plague in rats is the presence of a reaction at the site of inoculation in the majority of cases where the organism is introduced subcutaneously, and in about a third of the cases where the infectious material is rubbed on the shaven skin (cutaneous inoculation). The local reaction may exist only as a yellowish-brown crust overlying a granulating surface, and associated with a trifling thickening of the skin and subcutaneous tissue. It may appear as one or more firm papules 3 or 4 millimeters in diameter. The most frequent appearance is a brawny edematous and blood-stained reaction, which extends over an area perhaps an inch in diameter; at times purulent change may be well advanced. Very rarely one finds so extensive an edema as to cause the lesion to somewhat resemble the widespread gelatinous reaction seen so commonly in the guinea pig. Occasionally an extensive slough has been seen at the site of inoculation.

BUBO.

It is very exceptional that one finds in cases of induced plague the typical, firm, caseous bubo surrounded by an infiltrated area, as is so commonly seen in natural infection in rats. The glands are sometimes enlarged and injected without other changes. The commonest lesion, however, is a markedly enlarged gland which upon close inspection is seen to have a number of yellowish points just under the capsule. These points are especially well seen when a section is made through the gland. The gland may be squeezed out of the capsule, and it breaks down readily enough when pressure is made upon it, but the uniform necrotic process that one sees so often in natural rat plague is absent.

LIVER.

Granular lesions precisely like those found in natural infections are very common. If the rat has died on the sixth day or later, the ordinary lesions are apt to be replaced by necrotic foci that may be as much as 2 millimeters in diameter.

SPLEEN.

This organ is found mottled more frequently than in natural plague infection, and large granules are much more common.

The subcutaneous injection is rarely so well marked as it is in natural infections.

Pleural effusion of the same nature as that found in natural plague is common. Hemorrhagic foci are not rare in the lungs, and occasionally the organs are partly consolidated.

CHRONIC PLAGUE DUE TO ARTIFICIAL INOCULATION.

Occasionally a rat that has been inoculated but has survived a week or longer, will show, when killed, only an abscess at the site of the injection. Stained smear preparations may show a large variety of bacterial forms. We have not been able to demonstrate the presence of *B. pestis* in these lesions, yet there is no doubt but that the lesion is the result of the inoculation.

A lesion more frequently found is a caseous or a purulent lymphatic gland. If the inoculated rat has been killed about 10 days after the inoculation, in some cases one or more of the peripheral lymph glands will be found to be surrounded by an infiltration, and the gland itself will be purulent or less frequently caseous. Such lesions are occasionally met with in rats in which there is no suspicion of plague infection; but they are seen so frequently among rats that have survived artificial inoculation with *B. pestis*, there is no doubt but that in these cases they are the result of the inoculation. In several such cases pestlike organisms have been demonstrated in smears, and acute plague has been produced in guinea pigs by inoculation with the pus found in these lesions. Not infrequently in these cases the spleen will be found enlarged and looking very much like the organ in acute plague, but cultures from this organ in such cases have in my experience remained sterile.

In other cases the only lesions will be found in the spleen. The organ is enlarged and contains a number of caseous nodules. These nodules vary in number from 4 or 5 to 30 or 40 and in size from the head of a pin to a lesion 0.3 centimeter in diameter. In a number of such cases the nature of the lesion has been demonstrated by animal inoculation. For example, in a series of experiments carried out to determine the susceptibility of San Francisco rats to plague infection a large *Mus rattus* died on the eleventh day after inoculation. The post-mortem examination showed nothing except an enlarged spleen which contained about a dozen caseous nodules, the largest of which was not over 2 millimeters in diameter. The nodules were very firm and the capsule smooth, so that they were held with difficulty with dressing forceps. Cultures from the liver and the spleen remained sterile, but a piece of the spleen was placed beneath the skin of a guinea pig. This animal died of acute plague, and a pure culture of *B. pestis* was isolated from its liver. In some of these cases the liver will show large, distinct, whitish caseous foci. In another case a small *Mus norvegicus* was killed on the twelfth day after a cutaneous inoculation from an artificially infected squirrel. No lesion was found except in the spleen which was not materially enlarged, but which presented two small whitish caseous granules on the surface, neither being over 1 millimeter in diameter. A piece of the spleen containing one of these granules was put under the skin of the belly of a guinea pig. The guinea pig died on the fourth day with the usual lesions of acute plague. Occasionally in these cases of chronic plague punctate hemorrhages or even areas of consolidation are found in the lungs.

THE HISTOLOGY OF RAT PLAGUE.

Recent and satisfactory work on this subject is that of Ledingham (14), who has studied the lesions of both natural and induced plague in rats. The following is a very brief abstract of his work. The reader is referred to the original for a full study of the subject.

NATURAL RAT PLAGUE.

Two groups of cases are distinguished. First, those in which a large number of *B. pestis* are found in the liver and in the spleen. In the spleen this is accompanied by hemorrhages and congestion of the pulp sinuses and in the liver with congestion of the capillaries. These are early cases.

In the second group, or the later cases, there are extensive reaction changes in the tissues. In the spleen this leads at times to distinct abscess formation, but more frequently to a walling off of the foci of necrosis. In the liver more or less focal necrosis is found; sometimes the areas of "necrosis" may be so extensive that little healthy liver tissue remains. Bacilli are usually to be demonstrated in these areas of necrosis. Giant cells of the Langhans type may be found in the neighborhood of these foci.

The granular appearance of the liver is attributed to "hemorrhages and the focal necroses, together with the fatty changes in the liver cells. It must be understood, however, that a peculiar honeycomb-like vacuolar degeneration of the liver-cell protoplasm was far more frequent than any actual, coarse, fatty infiltration. The granular appearance of the spleen is due partly to endothelial catarrh and partly to subcapsular changes."

In experimental rat plague Ledingham found the lesions to resemble those of the first group of cases referred to above. There is usually marked bacteremia; focal necroses of the liver are scanty.

In a chronic case, minute abscesses were found scattered through the spleen. In the center of the abscesses were found clumps of degenerated bacilli. The areas were walled off by epithelioid and spindle cells and numerous giant cells of the tubercular type.

IMMUNITY OF RATS.

Contrary to the general impression the wild rat is not an animal especially susceptible to plague infection. The Indian Plague Commission (19) found that when rats are inoculated by the cutaneous method from the spleen of infected rats 59 per cent are immune to infection. A series of experiments conducted in the Federal laboratory in San Francisco showed that when inoculated with highly virulent cultures of *B. pestis* there is an immunity which is, however, more frequent among the large rats. When inoculated cutaneously with tissue containing large numbers of *B. pestis* from plague-infected human beings, rats, or squirrels, about 15 per cent of small rats and about 50 per cent of large ones were found to be immune. There is no good reason for believing that this immunity of San Francisco rats was due to a previous attack of the disease. Indeed, it was known beyond a doubt that some of the immune rats had never had an opportunity of becoming infected with plague in nature and thereby establishing an acquired immunity. We may mention here the fact that has been observed by many workers, and which we have amply confirmed, that rats are readily immunized by antiplague serum.

The subject of the transfer of infection directly from rat to rat by cutaneous or subcutaneous inoculation through a series of the animals is one that is evidently intimately associated with the preceding subject, as it is quite evident that an immune rat or several of them might terminate a series without any actual diminution in the virulence of the organism transferred. It is quite plain that the success of such an experiment would depend largely upon the number of rats used in each transfer. The Indian Plague Commission (19) had no difficulty in carrying infection through 26 transfers, using from 6 to 50 rats in each transfer.

Pound (7) in a series of eight experiments, was never able to convey the infection successfully beyond the sixth rat, using but one rat for each transfer. There was no apparent lessening of the virulence of the organism and each series appears to have been terminated abruptly by encountering an immune rat.

Baxter-Tyrie (15) says:

> It is probable that under certain natural circumstances a reduction in the virulence of the organism is effected and a comparative immunity is conferred on the rats. The infection of immigrant rats is, however, severe, and their arrival is heralded by a heavy mortality. In the same manner an infected rat imported into a fresh locality produces a similar result. This attenuation of virulence is responsible for the condition known as chronic rat plague.

Several experiments conducted in San Francisco to determine this point have given results that are regarded as showing merely the presence of a considerable percentage of immunity among the rats. It was observed that in each case certain of the rats died of acute plague even in the last transfer. It was very evident that had certain combinations of immune rats been encountered the experiment might have terminated at any point. On the other hand, by being especially fortunate in using nonimmune rats, the experiments might have given a much higher percentage of cases of acute plague. Unfortunately it was necessary to terminate these experiments in each instance before they could be regarded as completed.

The reason for the natural subsidence of plague among rats in any community is a point about which much more evidence must be obtained before we can speak with any degree of certainty. It may be due to the lack of susceptible material, possibly to a loss of virulence of the organism; but it seems more probable that it is due to a change in the number or relations of the ecto-parasites of the rat.

Adequate measures of rat extermination, while they may never bring about the ideal condition of a community that is free from rats, are, as is shown by the recent experience in San Francisco, of the utmost value in shortening the epizootic.

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SPECIMEN RAT RECORD CARD.

Legend: O=Ordinary; W=White belly; R=Red; Go.=Gopher rat; S=Small; M=Medium; L=Large M. R.=Mus rattus; M. N.=Mus norvegicus.]

No.	Date.	District No. 6.	Sex.		Size.			M. N.				M. R.		Pregnant.
			M.	F.	S.	M.	L.	O.	W.	R.	Go.	O.	W.	
19	Dec. 10, 1908	401 Fillmore Street....	1	....	....	1	....	....	....	1	....	......	......	....
20	.....do.......	.....do...............	....	1	....	....	1	1	....	....	....	......	......	[1]7

[1] Number of fœtuses.

SPECIMEN PLAGUE RAT CARD.

PLAGUE RAT NO. 50.

Date: June 20, 1908.
Species: *M. norvegicus*.
From District No. 6, sewer, Haight and Steiner Streets.
Condition: Badly injured by trap; thorax crushed.
Subcutaneous injection: General, marked.
Lymphatic glands, bubo or other lesions: Right inguinal bubo, caseous.
Liver: Typical whitish granules.
Spleen: Large, dark, firm.
Pleural effusion: Unable to say.
Purulent or caseous foci:
Diagnosis from gross lesions: Plague.
Diagnosis from smears: Plague (spleen and bubo).
Cultures: *B. pestis* recovered from liver culture.
Inoculation, guinea pig No. 50 A, +6.25.08.
Vaccination, guinea pig No. 50 B, +6.26.08.
Date suspicious:
Date positive: June 20, 1908.
Date negative: